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Effect of Isolated Soy Protein on the Bioavailability of Copper in Ground Beef.

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EFFECT OF ISOLATED SOY PROTEIN ON THE BIOAVAILABILITY OF COPPER IN GROUND BEEF

A Dissertation

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requirements for the degree of
Doctor of Philosophy

in

The Department of Food Science

by
James L. Smith
B.S., Ohio University, 1964
M.S., The Ohio State University, 1966
December, 1983

DEDICATION

THIS WORK IS DEDICATED TO

MY FATHER, JAMES

TO

JIMMY, NENA, KIKA, MARGIE,

MARCIE AND MIKEY

TO

MY WIFE, REINA

AND TO

THE MEMORY OF MY MOTHER,

SARAH

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Test diets containing ground beef extended with 0, 10, 20 and 30% ISP were fed to Single Comb White Leghorn chicks and 28 days to determine the effect of isolated soy protein (ISP) on the availability of copper naturally present in meat.

Copper content of the test diets decreased with increasing levels of ISP, ranging from 6.89±0.17 ppm to 2.09±0.12 ppm. Mean feed consumption per diet also decreased with increasing ISP levels as did the amount of copper consumed per bird. Although the mg copper consumed per bird decreased from diet I to diet IV, the mean liver copper values decreased from 1.48 ppm for the first diet to 0.61 ppm for diet III but increased to 1.03 ppm for diet IV. The calculated availability of natural copper in the diets increased from a low of 21.86% for diet I to 74.19% for diet IV. This high availability of copper from diet IV was probably due to an increased absorption of copper and larger liver weights in these animals. It appeared that the ISP had little or no negative effect on the availability of copper in the diets.

Mean copper values of uncooked ISP extended meats by the in-vitro method decreased with increasing levels of ISP. Copper content of the in-vitro diets decreased also as did the percent copper transferred across the dialysis membrane. A slight binding of copper by ISP may occur. Cooking the meat mixture reduced the amount of copper crossing the semipermeable membrane.

Electrophoresis of water and salt soluble proteins from chick duodenum showed high molecular weight proteins, none of which contained measurable amounts of copper. High molecular weight proteins also resulted from the electrophoresis of water and salt soluble proteins from chick ileum. Mineral analysis of the protein bands showed copper to be associated with two high molecular weight water soluble proteins and one high molecular weight salt soluble protein.

INTRODUCTION

Meat has always been important in the human diet, even though early man did not understand why such diets produced general health and well being. However, we now know that meat is a concentrated source of many nutrients such as proteins, certain fats, many vitamins and minerals, among which are iron, zinc and copper. Meat has long been a favorite of the housewife because of its economy, ease of preparation, and because it is nutritious. However, due to fluctuations in the world economy in recent years, the price of meat has tended to increase. In an attempt to help alleviate this problem, it was found that ground beef could be extended by the addition of soy protein, thus reducing its cost, but without an accompanying decrease in nutritional value. This practice is now very common commercially.

With the discovery that some components of vegetable material, such as fiber, phytates and oxalates are capable of chelating and binding certain vitamins and minerals, it has become necessary to reassess the use of soy protein as extenders of meat products. It has been reported that isolated soy protein is capable of altering the absorption of iron, zinc, copper and other minerals from different foods.

Zinc and iron are important in human as well as in animal nutrition, and since the 1920's copper has been known to be an important and essential component of the diet as well, so much so, that certain anemias will not respond to iron unless copper is present. In view of this, it is interesting to note that a great

majority of food analysis reports treat copper as being secondary in importance to zinc, iron and other elements. There have been few studies in which copper has been the primary focus of attention.

This study was due to determine what effect isolated soy protein, substituted in ground beef at different levels as an extender of the meat, might have on the copper naturally present in the product. This study was carried out by both in-vivo and in-vitro methods.

LITERATURE REVIEW

Metals have played an important role in the development of human society. Gold and silver were the first metals to be discovered but were primarily used as jewelry and ornaments to demonstrate the wealth of their owners. The discovery of copper, which followed that of gold and silver, allowed the beginning of a new age in the development of mankind. Copper was alloyed with other metals such as zinc, zinc and nickel, and with tin to form brass, nickel silver and, perhaps most importantly, bronze. Copper then perhaps represents the first metal to be used by man for practical purposes (55).

By the beginning of the Christian era, copper compounds were commonly used in the treatment of mental, pulmonary and other diseases. This use of copper, usually unsuccessful, was popular even up to and during the 19th century. During this century copper was recognized as a normal constituent of blood (55). However, this metal was not thought to be an essential part of the diet of either humans or animals until the reports of Hart, et al. (37) and Waddell, et al. (86) which indicated that the extracts or ash of essentially iron-free plant materials such as corn meal or chlorophyll, favored the assimilation and utilization of iron in hemoglobin building in rabbits fed a whole milk diet. The classic studies of Hart, et al. (38) showed that milk-fed rats developed an anemia that responded to iron only if copper was added. Other studies (22, 23) demonstrated that the same conditions existed in pigs and chicks.

BODY COPPER

The body of a normal adult contains approximately 100 to 150 mg of copper (79). The brain and liver contain much higher concentrations of copper than other organs and tissues, with about 8 mg each (55). Bovine liver has been found to contain approximately 100 µg copper/g of tissue (52). The elevated content of copper in the liver is related to its storage function and also the liver is the only site of synthesis and release of ceruloplasmin (55). Copper in the liver is primarily associated with copper-binding proteins and metalloenzymes (79). The kidney, heart and spleen contain progressively less copper (11). The normal fetus at term contains about 100 mg of copper with the distribution being very different from that in the adult. During fetal life, the percentage of copper in the body increases progressively until at birth the liver contains 6-10 times the concentration in the liver of the adult (55, 79).

Besides its function of copper storage and ceruloplasmin synthesis, the liver serves as the major pathway of copper excretion via the biliary tract and also releases copper to maintain the labile copper pool in the serum and plasma. Copper from this pool is incorporated into superoxide dismutase or other copper-containing enzymes of body tissues (55). The ceruloplasmin synthesized in the liver is released so that is comprises more than 90% of plasma copper, which remains constant in healthy adult mammals (55, 67). Evidently there is no interchange in the blood stream between other forms of copper and ceruloplasmin copper (78).

The measurement of copper in serum or plasma has undergone many changes from an acidification of the blood samples to release the

copper and react with diethyldithiocarbamate (55), to enzymatic determinations (43, 69, 70) to immunological methods (56). Due to the multiplicity of methods for estimating copper and ceruloplasmin, values for serum, whole blood and plasma vary greatly (55). Blood levels of copper are generally expressed as serum or plasma levels with little distinction between the two. However, Rosenthal and Blackburn (71) report significant differences between serum and plasma levels. Values range from 106.2 to 106.9 µg/100 ml in blood serum for males and females respectively (1) to 105±16 µg per 100 ml for males and to 116±16 µg/100 ml for females (91). Normal adult serum contains about 30 mg of ceruloplasmin per deciliter, although values vary according to the method used (67). However, even with this variation it is clear that women not on oral contraceptives have somewhat higher levels than do men (19, 91). Full-term infants have very low ceruloplasmin levels at birth but reach adult levels by 3-6 months of age (67).

The concentration of ceruloplasmin varies widely with the animal species (67). However, increases in the serum level with age has been demonstrated in rats (88) and pigs (13). The serum ceruloplasmin in the rooster also increases with age. When the animal weighs 125 g it has 8% of the adult levels, 16% at 200 g, 20% at 315 g, 31% at 475 g and 46% at 900 g. Adult levels are reached at 2,100 g. These levels represent about one-tenth the levels in man (68).

COPPER PROTEINS

Copper is an integral part of a number of proteins. It may be Cu^{+} or Cu^{++}, or both, with shifts back and forth during enzymatic

reactions. Copper-containing proteins may be classified as oxygen-carriers (hemocyanin), oxidizing catalysts (ascorbate oxidase) and those whose functions are unknown (67).

Amine oxidases

There are three general types of amine oxidases. The first of these, monoamine oxidase, is a family of enzymes which contain copper. In general, they have molecular weights of 290,000 daltons in bovine and rat liver and 64,000 in human liver (67). It seems that these oxidases act in the deamination of norepinephrine, serotonin and histamine (55, 67, 78). The enzyme contains from two to eight atoms of copper per mole (67, 79). However, in some enzymes it appears that the copper may not be necessary for catalytic activity (24). Other metals such as zinc may be present in these enzymes (67).

Diamine oxidase is a copper-containing enzyme whether isolated from pig kidney, bovine plasma or from pea seedlings. It contains two atoms of copper per mole in the cupric form and is capable of oxidizing histamine, cadaverine, putrescine and other similar substances. In pig plasma, this enzyme has been called benzylamine oxidase or histaminase, although the enzymatic activity toward benzylamine is 100 times greater than toward histamine. This copper-containing enzyme is pink and has a molecular weight of approximately 190,000 (55, 67). The third amine oxidase, lysyl oxidase, was discovered from severely copper deficient states and lathyrism induced by various agents. In both conditions, lysyl oxidase activity in connective tissue and arterial walls is reduced (67). The major function of this enzyme is to catalyze the oxidative deamination of the ε-amino groups of peptidyl lysine or hydroxylysine to form

α-aminoadipic-δ-semialdehyde derivatives as a first step in the cross-linking of immature elastin and collagen into satbile fibrils (6, 54, 55, 67). The enzyme has been shown to be important in the formation of eggshell membrane (4, 36).

Ascorbate oxidase

This is a blue protein, with a molecular weight of 140,000 to 150,000 daltons and contains 8 to 10 copper atoms, two of which are thought to be Cu^{+} while the remainder appear to be Cu^{++}, and are the only ones necessary for enzymatic activity. This enzyme catalyzes the oxidation of ascorbate to dehydroascorbate (55, 67, 79).

The copper-containing enzyme carboxypeptidase A also catalyzes the oxidation of ascorbic acid (67).

Cytochrome c oxidase

Cytochrome c oxidase is the last step in the electron transport chain. It is found primarily in the mitochondrial membrane rather than in the cytosol (73, 78). The copper is found in the redox centers of the enzyme (67, 90).

Dopamine β-hydroxylase

Dopamine β-hydroxylase is a colorless enzyme whose major source is the adrenal glands. It has a molecular weight of 290,000 in the bovine (32), has from four to eight copper atoms per mole, and catalyzes the hydroxylation of dopamine to norepinephrine (55, 67, 79).

Superoxide dismutase

Probably one of the most widely studied enzymes, superoxide dismutase (SOD) is found primarily in erythrocytes and cytosol of eucaryotic cells. It is a acavenger of the superoxide and is believed to catalyze the conversion of this ion (O_2^-) to H_2O_2 and O_2. The H_2O_2 formed is further removed by catalases and peroxidases (55, 67, 79).

One type of SOD contains both copper and zinc. It appears that copper has a functional role while zinc has a structural role (31, 78). Another SOD contains iron while still another contains manganese (67). The copper-containing enzyme generally contains two copper atoms per molecule (79). The concentration of SOD varies in different tissues, between species, and in different pathological conditions (67).

There are also other copper proteins such as hemocyanin, luciferase, tyrosinase and uricase (55, 67).

Ceruloplasmin

One of the most important copper proteins is ceruloplasmin. This is the blue copper protein of normal plasma which has ferrooxidase activity (42, 78, 79). The blue color is due to the cupric form and is not associated with molecular oxygen. Thus, copper is important for both its color and its oxidative action (5). Ceruloplasmin has a molecular weight of 124,000 to 143,000 daltons and contains 6-7 atoms of copper per mole (67, 79).

At present it is fairly well established that ceruloplasmin is essential for mobilization of stored iron (65, 68). Copper deficient pigs take up iron in the intestinal mucosa but it is not absorbed. It

would therefore appear that the ceruloplasmin is necessary for release of iron not only from liver stores but from other stores as well (67). It has been shown that isolated livers release iron if the perfusing medium contains ceruloplasmin (46, 66).

Besides its ferrooxidase activity, ceruloplasmin is thought to function also as a copper transport protein and in the oxidation of a number of biological amines such as dopamine, adrenaline and serotonin.

ABSORPTION AND TRANSPORT OF COPPER

The absorption of copper seems to be regulated at the intestinal mucosa. Although the site of maximal absorption varies among mammalian species, in man this occurs proximal to the third portion of the duodenum (55, 79). The efficiency of gastrointestinal copper absorption has been estimated at 30-50% (79). A recent study reported that two subjects fed 1.5 mg copper per day for 20 days, absorbed 85% and 70% respectively of a 2 mg ^{65}Cu dose on the 21st day after an overnight fast. The apparent copper retention ranged from -36% to +31% (45). Two mechanisms seem to be involved in copper absorption. The first of these is a passive diffusion while the second involves the absorption of copper- amino acid complexes and is an energy-dependent mechanism (67, 79). However, due to the fact that in a normal mixed diet much of the copper is present as copper-proteins and metalloenzymes, an absorption distal to the duodenum could be expected since a proteolytic digestion of these foods would seem to be necessary to release copper (79).

Availability studies with different salts have shown that copper is optimally absorbed in the SO_4 form (89).

Ionic copper is transported by albumin in the blood of mammals. In the normal mammal over 90% of the copper in the plasma is incorporated into ceruloplasmin while of the remaining 10%, 90% is attached to albumin and the rest to amino acids (18, 78). When the albumin-copper complex reaches the liver, copper is released to receptors on the hepatocyte cell membranes and is subsequently transferred to the cytosol where it is bound to metallothioneins (55). The copper-amino acid complexes pass from the blood to tissues either by an active transport or by simple diffusion (63).

The estimates of 30-50% absorption of dietary copper are based upon differences between intake and fecal excretion, since urinary excretion is very minor, accounting for only 30 to 60 μg of the total daily output. Fecal excretion consists of unabsorbed dietary copper, copper excreted via the biliary tract and the salivary glands (55). Bile is the principal means of copper excretion. Bile obtained postmortem contained an average of 0.329 mg copper per 100 ml. It has also been estimated that 0.5 to 1.3 mg copper is excreted daily in the bile (11), and is thought to be associated with large macromolecules (55, 79), or with the bile pigments such as bilirubin (57). It is not yet known whether fresh bile in the intestine will bind dietary copper, thus making it unavailable for absorption (79).

INTERACTIONS WITH OTHER ELEMENTS

Interrelationships between copper and other trace elements and substances such as zinc, iron, cadmium and ascorbic acid in mammalian

metabolism have been shown in animal studies. Copper and zinc have been shown to be mutually antagonistic. Copper has been shown to reduce the toxicity that may result from high dietary intakes of zinc in chicks. Also high dietary zinc can induce or aggravate a conditioned copper deficiency which then restricts iron utilization in chicks (39, 40). Moreover, increased intakes of dietary zinc have been shown to increase the tolerance of pigs to higher intakes of copper. The effects of high dietary copper can be accentuated that dietary zinc exerts its antagonistic effect on copper absorption by inducing the synthesis of a metallothionein in the mucosal cells which then sequesters the copper, making it unavailable for serosal transfer (28, 29).

The interrelationship of copper and iron has been widely studied (5, 41, 65, 68). However, there is a paucity of information on the effect of dietary cadmium on copper. Cadmium has been shown to be an antagonist of copper in chicks when fed in high concentrations. Its effect has been demonstrated by a significantly lower lung elastin content in these animals due to a decrease in lysyl oxidase levels (54).

Experiments on the interaction of copper and dietary nickel in rats suggest both a synergistic and an antagonistic interaction between these two elements (62, 80).

Ascorbic acid reduces the intestinal absorption of copper. It has been shown that a high ascorbic acid intake (500 mg with each meal for 65 days) is antagonistic to the copper status of man (26). The mechanism for this negative effect of ascorbic acid on copper metabolism appears to be largely one of gastrointestinal absorption.

It has been hypothesized that the lumenal ascorbate in rats reduces the absorbable Cu^{++} to the less absorbable Cu^{+} (84). The antagonistic effect of high dietary levels of ascorbic acid to the metabolism and function of copper has also been demonstrated in chickens (10, 41), guinea pigs (75) and rabbits (44).

Several studies in rats have suggested that a deficiency of copper or an elevated zinc:copper ratio in the diet would raise circulating cholesterol levels (1, 2, 27, 47). Other studies have shown that the antagonistic effect of high ascorbic acid on the intestinal absorption of copper makes this unavailable for regulating cholesterol metabolism allowing an increase in serum cholesterol (49, 59). Several studies have suggested that a high zinc to copper ratio may result in hypercholesterolemia and thus in an increased risk of coronary heart disease (2, 48, 49).

Oral contraceptive agents have been shown to produce a significant increase in serum copper levels in young women after three months. There does not appear to be an effect of these agents on levels of zinc and copper in hair (16, 85).

CLINICAL MANIFESTATIONS OF HUMAN DEFICIENCY

Copper is ubiquitous in nature and is found in almost all types of food. It would therefore be logical to expect that mixed diets would contain sufficient copper to prevent deficiencies. Many statements have been made to the effect that due to this ubiquity of copper and due to a lack of recognized manifestations of copper deficiencies in man such as those observed in artificially or naturally depleted animals, man appears to be free of the problems associated with a

state of copper deficiency. However, as a result of rather special types of nutritional situations, such as in infants recovering from marasmus or kwashiorkor being fed milk diets, and in persons receiving total parenteral nutrition (TPN) without copper supplementation, evidence for the presence of copper deficiency in man has developed (55, 78). Some infants fed diets limited largely to milk have been shown to develop hypocupremia, hypoferremia, hypoproteinemia and hypochromic anemia which is responsive to oral copper but not to iron (53). Copper deficiencies characterized by neutropenia, hypoceruloplasminemia and osteoporosis have been reported in premature infants. Oral copper generally elicits a favorable response (15, 53).

Neutropenia and hypochromic anemia which responds to oral copper but not oral iron are clearly signs of deficiency. This deficiency is due to lowered ceruloplasmin levels and an impaired release and transport of iron from body stores. Skeletal demineralization results from a deficiency of copper-containing oxidases which are essential for the cross-linking of bone collagen (42, 55). A decreased skin depigmentation is another common characteristic of the copper deficient infant, which might be attributed to a decreased activity of tyrosinase, a copper-containing enzyme necessary for the production of melanin (67). In the latter stages of deficiency, neurological disorders such as hypotonia may be produced by decreased levels of cytochrome c oxidase (55, 78).

It is interesting to note that many of the reported copper deficiency symptoms are similar to those reported for Menkes' steely-hair syndrome. This is a progressive brain disease which is inherited as a sex-linked recessive trait. It has been referred to as

trichopoliodystrophy and, more commonly, "kinky-hair" disease. "Steely-hair" appears to be a more appropriate term, since the hair more closely resembles the depigmentation and loss of crimp in wool observed in copper-deficient sheep (33, 78). Symptoms usually appear between birth and three months of age, with death resulting by the fourth or fifth year (55).

Nutritional copper deficiency and Menkes' disease have several features in common: 1) they usually occur in infancy; 2) hypocupremia and hypoceruloplasminemia; 3) defective elastin formation resulting in arterial aneurysms and a decreased tensible strength of skin; 4) changes in long bones similar to those of scurvy; 5) decreased pigmentation of skin and hair (42, 55, 79). However, Menkes' disease differs from the deficiency state in the formation of pili torti (kinky hair), mental retardation and cerebral and cerebellar degeneration, hypothermia and an absence of anemia and neutropenia (55, 79). Reduced absorption of copper is an important factor in the disease. Interestingly, the excretory loss of copper is reduced and the retention time of most of the copper is increased, thus effectively increasing the biological half-life in the body (55, 67, 79). There also appears to be a defect in the intestinal transport of copper (55, 67).

Some pathogenetic factors that might contribute to human copper deficiencies are listed in Table 1.

COPPER IN THE DIET

It is well known that copper is present in almost all types of food, although levels vary greatly depending on the soils upon which

Table 1. Some pathogenetic factors in human copper deficiency.

I.	Decreased intake
	1. Low copper infant formula
	2. Total parenteral nutrition without added copper
II.	Decreased absorption
	1. Iron-fortified milk formulas
	2. Megadose or high zinc intake
	3. High ascorbic acid intake
III.	Decreased utilization
	1. Menkes' steely-hair syndrome
IV.	Increased loss
V.	Increased requirements

they have been grown and on contamination before and after reaching market. Liver, especially from calf, lamb and beef, constitutes the richest source of copper in the human diet, followed by shellfish, especially oysters. Other foods contain lesser amounts, with milk having quite low levels of copper. Human milk contains 0.04 to 0.3 mg copper/100 ml while cow's milk has a mean of 0.086 mg/liter with a range of 0.04-0.19 mg/liter (60).

The dietary intake of copper in different countries varies principally due to variations in the diet. Most western-style mixed diets provide from 2-4 mg copper per day. In India, where the consumption of wheat and rice is quite high, copper intake has been estimated to be as much as 4.5 to 5.8 mg/day (55, 79). In the United States, a study of twenty diets developed by the USDA estimated mean copper intake to be 1.05 mg per day (50).

In RDA for copper in the U.S. population has not been established, although formerly 2 mg/day was widely discussed as the recommended allowance. However, the newest edition of the NRC-RDA mentions ranges based on age (Table 2) (63). A recent reports suggests that a mean copper intake between 1.24 and 1.35 mg from a mixed American diet will meet average requirements (51).

The average copper content of American infant diets was 1.87 mg (8). Soy-based meat analogs had a mean copper content of 0.09 mg/100 g while meat had an average copper level of 0.32 mg/100 g (79). In a diet adjusted for 100% of the recommended daily protein intakes, breast-fed infants would ingest 44% of the estimated adequate copper allowance, while adults would consume only 37% of the estimated safe intake. Adjusting the diet to 100% of the recommended dietary energy

Table 2. Estimated safe daily dietary intake of copper (63).

Age (yr)	Intake (mg)
0 - 1.0	0.5 - 1.0
1 - 6	1.0 - 2.0
7 - 10	2.0 - 2.5
11 - 18	2.0 - 3.0
19 +	2.0 - 3.0

allowance for the respective age groups, human milk would provide 33% for infants while adults would obtain 66% of the recommended copper from the mixed diets (79).

BIOAVAILABILITY OF COPPER

Reports of the influence of dietary fiber intake on mineral and trace element availability are not always in agreement (83). Few of the studies reported have shown a significant effect on the absorption of copper by phytates, dietary fiber or various fiber subcomponents (78, 83). A recent study has shown that copper in the presence of phytate alone produces a minimal precipitate. In other words, the interaction of copper and phytate was minimal. However, in the presence of calcium, the maximum precipitate was produced at pH 6.0, with 74% of the copper, 38% of the calcium and 26% of the phytate in the reaction being found in the resulting precipitate, thus demonstrating that phytate may affect the bioavailability of several minerals (64). It has also been shown that textured vegetable protein substituted in a casein-gelatin diet at a 25% level, produces lower manganese and copper levels in the liver (81).

Other factors besides dietary fiber and phytate affect the bioavailability of copper. Ascorbic acid, sulfates and sulfates cause a lower absorption of copper in animals, possibly by forming complexes of very low solubility (59, 79). Recent reports have shown that subjects fed a high calcium-high protein diet (2382 mg Ca, 2442 mg protein/day) lost more iron and copper when compared to that retained when fed a moderate calcium-moderate protein or a moderate calcium-high protein diet (77). It has been demonstrated that other minerals

such as iron and zinc competitively reduce copper absorption (23, 28, 78, 79), while manganese and nickel have been reported to be antagonistic to copper, thus lessening the apparent absorption of this mineral (62, 72, 88).

Meat and meat products are some of the best sources of zinc and copper in a highly available form. Nevertheless, high fiber-high carbohydrate components used to extend meats, or the presence of these components in other foods, readily chelate these elements (17). It has also been shown that copper availability from undenatured animal proteins is also reduced when compared to denatured animal proteins (79).

Several methods have been employed to determine copper levels in biological materials, among them atomic absorption spectroscopy, spectrophotometric techniques and neutron activation analysis. The spectrophotometric and atomic absorption determinations are easily and rapidly carried out, but results obtained with atomic absorption seem to be more accurate (74). Neutron activation analysis is the most accurate technique but is the method of choice only if no other methods are available (9).

Bioavailability of trace minerals in foods is routinely determined by feeding trials, employing farm or laboratory animals (10, 41, 59, 75). However, these in-vivo bioavailability studies have some disadvantages, principally, the relatively long time necessary to complete the trials. An in-vitro method which would simulate physiological conditions and, at the same time, shorten considerably the time required for the determinations would be preferred. Many investigators have utilized everted duodenal segments taken from

animals and tied into sacs for in-vitro studies (28, 29, 84). A recent report indicates that a simulated gastro-intestinal digestion using a semipermeable membrane may be useful for the in-vitro estimation of iron availability from meals (58). However, in-vitro determinations of copper have not been reported.

MATERIALS AND METHODS

Ground beef for the in-vivo and in-vitro studies was obtained from the Meats Laboratory of the Department of Animal Science of the Louisiana State University. The ground beef was frozen until utilized. The isolated soy protein used in this study was Supro 620, obtained from the Ralston Purina Company, Protein Division, St. Louis, MO. Reagent grade chemicals were used in all analyses. Distilled and deionized water was used exclusively throughout the study. All glassware was soaked overnight in chromic acid and rinsed with deionized water before use.

PROXIMATE ANALYSIS

The ash, moisture, fat and protein contents of the ground beef, isolated soy protein and all diets were determined in accordance with A.O.A.C. procedures (3). Protein determinations were done utilizing the Technicon Autoanalyzer II, equipped with a micro-kjehdahl cartridge.

IN-VIVO DETERMINATION

The in-vivo determination of copper bioavailability consisted of a 14 day depletion period followed by a 28 day feeding period with the test diets. During the 14 day depletion period all of the experimental animals consumed a semisynthetic diet deficient in copper. This copper deficient diet was found to contain 1.211 ppm copper. Preliminary feeding trials demonstrated that feeding the

copper deficient diet for 14 days produced a 25% reduction in liver copper levels.

Animals

Animals used were day-old Single Comb White Leghorn chicks obtained from the Department of Poultry Science of the Louisiana State University.

During the first 14 days of the trial, the chicks were randomly divided into five groups of 15 animals each. They were housed in raised cages equipped with stainless steel feeding troughs and glass watering bottles with plastic bases, all of which were acid washed and rinsed with deionized water. Distilled deionized water was fed throughout the 42 day period.

Upon initiating the 28 day feeding period, 15 animals were slaughtered and the livers analyzed for total copper content. The remaining animals were randomly assigned to different cages, five animals per cage. These were then randomly assigned to treatments, with three cages per treatment. As before, each cage was equipped with stainless steel feeding troughs and glass watering bottles.

Diets

The copper deficient diet utilized was the purified diet for chicks reported by Cerniglia (12) (Table 3), modified by the elimination of the $CuSO_4.5H_2O$ from the mineral mix (Table 4). The copper content of this diet was found to be 1.211 ppm. This diet was offered to the experimental animals ad-libitum during the 14 day depletion period.

The experimental diets consisted of ground beef extended with isolated soy protein at levels of 0, 10, 20 and 30% (Table 5). The isolated soy protein (ISP) was rehydrated before addition to the meat by adding water at a level five times the weight of the ISP. This rehydrated ISP was then added to the ground beef at the levels previously described. The trace mineral levels of each of the diets were adjusted to conform to the levels recommended by the Animal Nutrition Research Council (61), with the exception of copper. The levels of copper in the diets were those naturally present in the meat-ISP mixtures. Likewise, the protein levels were those supplied naturally by the components of the diet. The energy level was adjusted to be as nearly isocaloric as possible.

All experimental diets were dried in a forced-air oven (Blue M, Blue Island, IL) at 80°C for 10 hours. After cooling, the vitamin pre-mix (Table 6) was added to the dried feed. The feed was then ground and stored in plastic bags at 4°C. During the feeding trials the feed was offered ad-libitum. The total amount of feed consumed was determined at the finalization of the 28 day trial period.

MINERAL ANALYSIS

In order to determine the availability of copper, the ceruloplasmin level in the serum is commonly monitored. An attempt was made to measure the ceruloplasmin levels in the serum of chicks by the method of Houchin as modified by Rice (70). In preliminary studies, it was impossible to detect ceruloplasmin in the chicks. Apparently, the level of ceruloplasmin in these animals, even up to six weeks of age, was below the detection limits of this method. It was therefore

Table 3. Purified diets for chicks. (From 12)

Ingredient	%	g/kg
Glucose	20.00	200.00
Cornstarch	20.00	200.00
Isolated Soy	30.00	300.00
Cellulose	12.00	120.00
Mineral Mix-Purified	6.00	60.00
Vitamin Mix-Purified	1.00	10.00
Refined Soybean Oil	10.00	10.00

Table 4. Mineral mix for the purified diet (12).

Ingredient	Grams
$CaCO_3$	15
$Ca_3(PO_4)_2$	14
K_2HPO_4	9
Na_2HPO_4	7.2
$MgSO_4.7H_2O$	5.0
KI	.05
$Na_2MoO_4.2H_2O$	.01
$MnSO_4.H_2O$	.40
$ZnCO_3$	.20
Fe cit.$5H_2O$	.50
$CuSO_4.5H_2O$	.016
Na_2SeO_3	.0002
NaCl	5.0
Sand	3.6238
Total	60.00

Table 5. Composition of diets used in the feeding trial.

Diets	Ingredients (%) Ground beef	Isolated soy protein
I	100	0
II	90	10
III	80	20
IV	70	30

Mineral mixture to provide 15 g $CaCO_3$, 14 g $Ca_3(PO_4)_2$, 9 g K_2HPO_4, 7.2 g Na_2HPO_4, 5 g $MgSO_4.7H_2O$, 0.05 g KI, 0.02 g NaBr, 0.01 g Na_2MoO_4, 0.4 g $MnSo_4.H_2O$, 0.2 g $ZnCO_3$ and 0.5 g ferric citrate per kg of feed.

Vitamin pre-mix - 10 g/kg dried feed.

Table 6. Vitamin pre-mix used in the purified diet of Cerniglia (12) and in the test diets.

Ingredient	g/kg	amount/kg diet at 1%
Vitamin concentrate	100.00	1 g
Choline Chloride	200.00	1400 mg
DL-Methionine	600.00	6 g
Glucose	100.00	1 g
Total	1000.00 g	

decided to employ the measurement of total liver copper for determining the repletion of copper in animals fed the test diets.

Sampling

At the termination of the feeding period, the animals were weighed, sacrificed by cervical dislocation and the livers removed. Each liver was washed with distilled deionized water to remove all copper-containing contaminants, blotted, weighed and frozen until they were to be ashed.

Ashing

In order to determine the copper content of the livers, these were first wet ashed using 5 ml 9N HNO_3 per gram of sample. The mixture was then heated almost to dryness and then diluted to 25 ml with 0.1N HNO_3.

Mineral determination

Total copper content of the ashed liver samples was determined by inductively coupled argon plasma spectrophotometry (ICAP) (25), in the Department of Agronomy of Louisiana State University, using a Model 34100 Inductively Coupled Argon Plasma Quantometer (Applied Research Laboratories, Sunland, CA), equipped with a Digital Decwriter II output printer.

IN-VITRO DETERMINATION

DIETS

The diets tested by the in-vitro method were ground beef extended with different levels of the rehydrated isolated soy protein to 0, 10,

20 and 30%. It was unnecessary to adjust the mineral, protein or vitamin content of the diets.

To determine the effect of cooking on the cooper in the meat-ISP diets, each was cooked in one of two ways. A portion of each diet was cooked in a microwave oven (Amana Radarange) for five minutes/300 g of meat. A separate portion of the same size was pan fried until well done.

The availability of copper in the raw uncooked diets, as well as in cooked samples, was determined by the method reported by Miller, et al. for iron in meals (58).

Reagents and materials

Pepsin - Sixteen grams of pepsin (Sigma Chemical Co., St. Louis, MO) was first suspended in 0.1N HCl and then brought to 100 ml with 0.1N HCl.

Pancreatin-bile - 25 g pancreatin (porcine, Sigma Chemical Col, St. Louis, MO and four grams bile extract (porcine, Sigma Chemical Co., St. Louis, MO) were suspended in an aliquot of 0.1M $NaHCO_3$ and the mixture was brought to one liter with 0.1M $NaHCO_3$.

Dialysis tubing - Tubing with a molecular weight cutoff of 6000 to 8000 was used throughout.

Sample preparation

Since the in-vitro determination is designed to simulate natural digestion, the test meals were first homogenized in a food blender to a smooth creamy consistency, after which the pH was adjusted to pH 2 with 6N HCl to simulate stomach conditions in the animal. The samples were divided into aliquots and frozen in plastic bags at -20°C.

Pepsin-HCl digestion

Samples of the frozen homogenized test diets were thawed at 37°C in a shaking water bath. The pepsin solution was then added in sufficient quantity to provide 0.5 g pepsin/100 g of food. The pepsin was mixed with the food and the entire mixture incubated at 37°C for two hours in a shaking water bath. After incubation, the digest was divided into 20 g aliquots, one of which was used for the titratable acidity determination, while the others were frozen at -20°C.

Titratable acidity

Titratable acidity of a 20 g aliquot of the pepsin digest, to which 5.0 ml of the pancreatin-bile mixture was added, was determined by titration with 0.5N KOH.

Pancreatin digestion

The following step of the simulated digestion involved a further digestion by a pancreatin-bile extract mixture of the material resulting from the digestion with pepsin.

Aliquots (20 g) of the pepsin digest were thawed and transferred to 100 ml beakers into which were then placed segments of dialysis tubing which contained an amount of 0.5N $NaHCO_3$ equivalent to the titratable acidity determined previously, and 20 ml distilled, deionized water. The beakers were then sealed with parafilm and incubated at 37°C in a shaking water bath for approximately 30 minutes, or until the pH of the mixture reached 5. At this point, 5.0 ml of the pancreatin-bile extract mixture were added and the incubation continued for another two hours. At the termination of the incubation period, the dialysis tubes were removed, rinsed with

distilled deionized water and the contents weighed. The dialysates were analyzed without further dilution for total copper by ICAP.

In an attempt to ascertain the type of protein in the intestine responsible for the binding of copper during its transport across the intestinal mucosa, duodenal segments from chicks fed diet I were taken at the moment that the livers were removed. Segments of the ileum were also removed and were flushed with distilled deionized water to remove all material not bound to the intestinal mucosa.

ELECTROPHORESIS

A modification of the dodecyl-polyacrylamide gel electrophoresis method described by Weber and Osborn (87) was used in this study.

Protein extraction

Both salt soluble and water soluble proteins were extracted using the method described by Dowdie (20).

Salt soluble proteins were extracted by homogenizing 2.5 g of duodenal tissue for two minutes at low speed and four minutes at high speed with 40 ml of chilled 5% NaCl adjusted to pH 7.1 with 0.5M $NaHCO_3$. 4.5 portions of ileum were also homogenized with 70 ml of the chilled 5% NaCl in the same manner. The homogenate was then mixed for 10 minutes on a magnetic stirrer and centrifuged at 8000 x G (3500 rpm) for 20 minutes using a Sorvall R-C2-B refrigerated centrifuge at 4°C. The supernatant was filtered with Whatman #42 filter paper. The centrifugate was then extracted two more times with successive 10 ml quantities of the chilled 5% NaCl solution, and were combined with the first extract.

The extraction of water soluble proteins was the same as the procedure previously described with the difference that chilled deionized water was employed instead of the 5% NaCl solution.

The protein concentration from each extraction procedure was determined by the Biuret test (34) using crystallized bovine serum albumin (Sigma Chemical Co., St. Louis, MO) for the standard curve.

Sample hydrolysis

A 1:1 dilution of the protein extract was prepared with a solution containing 4% sodium dodecyl sulfate (SDS) and 2% β-mercaptoethanol in 0.1M sodium phosphate biffer, pH 7.5. This solution was shaken for two hours at ambient temperature to affect the hydrolysis of the proteins present. The resulting material was used in the electrophoretic separation of the proteins.

Gel preparation

Pyrex glass tubes (13 cm x 6 mm I.D.) were soaked overnight in chromic acid and rinsed with deionized water followed by a rinse with photoflow (Eastman, NY). The tubes were then dried at 80°C in a forced air oven (Blue M, Blue Island, IL). After cooling, one end of the tubes were sealed with double layers of parafilm.

To prepare the gel, 3.75 g acrylamide and 0.1 g bis acrylamide were mixed in 50 ml 0.1M sodium phosphate buffer. The mixture was deaerated for approximately 20 minutes using a water aspirator. After deaeration, 0.8 ml of an ammonium persulfate solution (85 mg ammonium persulfate in 5 ml water) and 25 µl N,N,N',N'-tatramethylenediamine (TEMED) were added to the gel solution, which was the deoxygenated for another 2-3 minutes. The gel solution was then carefully transferred

with pasteur pipets to the pyrex tubes to a height of approximately 11 cm. The tubes thus prepared were carefully overlayed with water to prevent dehydration of the gel.

Sample preparation for electrophoresis

For electrophoresis separation of proteins, 90 µl of the hydrolyzed protein solution containing approximately 15 µg protein was placed in a small vial together with 10 µl of bromophenol blue tracking dye and three drops of glycerol. The mixture was transferred to the gel tubes with a pasteur pipet and the tubes were overlayed with phosphate buffer. The protein samples thus prepared were placed in an 18-tube capacity Bio Rad electrophoresis unit, Model 155, equipped with a Buchler 3-1500 power supply. Reservoir buffer was added to both upper and lower compartments and the electrophoresis unit connected to the power supply. The current was adjusted to 5 MA per gel tube. Current was applied continuously until the marker dye was approximately 1 cm from the bottom of the gel. The electrophoresis unit was disconnected from the power supply and the gels were removed from the glass tubes. Each gel was then cut at the leading front of the marker dye and were placed in test tubes containing a staining solution consisting of 0.25% Comassie Blue dye dissolved in 45% methanol and 10% acetic acid in water. The gels were stained overnight and then destained with a solution of 7.5% acetic acid and 15% methanol in water. The solution was changed periodically until the different bands could be easily distinguished. The gels were then stored in a 7.5% solution of acetic acid.

Standard Proteins

Standard molecular weight markers used in this study were α-lactalbumin (M.W. 14,200), soybean trypsin inhibitor (M.W. 20,100), trypsinogen (M.W. 24,000), carbonic anhydrase (M.W. 29,000), glyceraldehyde anhydrase (M.W. 36,000), ovalbumin (M.W. 45,000) and bovine serum albumin (M.W. 66,000). 30 µl of the standard mixture was added to each standard gel tube. The standard mixture was obtained from Sigma Chemical Co., St. Louis, MO.

Rm and molecular weights

After destaining of the gels, the total length of each gel was measured, as well as the distance from the top of the gel to the center of each band on the gel. The relative mobility (Rm) was calculated from the following equation:

$$Rm = \frac{\text{Distance of protein migration}}{\text{Distance of dye migration}}$$

Molecular weights were determined from the standard curve elaborated from Rm values of standard proteins (Figure 1). The protein subunits on each gel were quantified by means of photodensitometry at 590 nm.

Mineral analyses

Protein bands were cut from the gels, ashed with 9N HNO_3 as previously described and analyzed for copper by ICAP.

The experimental design utilized in this study was the completely randomized design described by Snedecor and Cochran (76) and Cochran

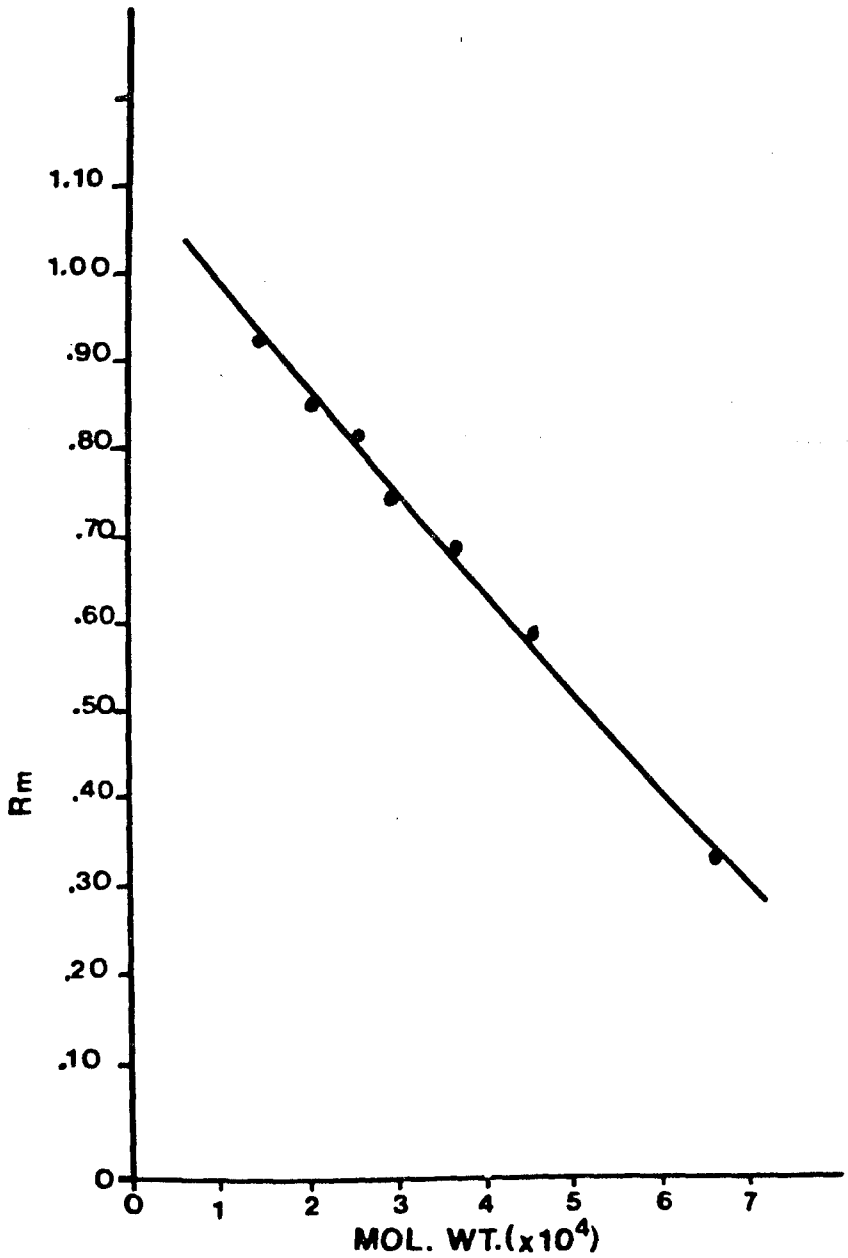

Figure 1. Sample standard curve used for the determination of protein molecular weights ($y=1.0687 + 1.1323 \times 10^{-5} x$).

and Cox (14). All data was analyzed by the general linear models procedure of SAS.

RESULTS AND DISCUSSION

Results of proximate analyses of the ground beef and isolated soy protein (ISP) used in this study are summarized in Table 7.

Table 7. Proximate analysis of ground beef and isolated soy protein.

	Percent (%)	
	Ground beef	Isolated soy protein
Ash	0.90±0.01	4.01±1.25
Moisture	61.84±1.56	6.05±0.78
Fat	14.7 ±1.33	0.37±0.24
Protein	22.4 ±1.96	86.2 ±2.01

IN-VIVO STUDIES

The levels of copper and protein as determined on the moisture-free test diets are reported in Table 8. This table summarizes the data in Table I of the appendix. Copper levels were those naturally present in the feed as were the levels of protein. Copper ranged from a high of 6.89±0.17 ppm, or .00689 mg of copper per gram of feed, for diet I (0% ISP) to a low of 2.09±0.12 ppm, or .00209 mg of copper per gram of feed, for diet IV (30% ISP). No attempt was made to adjust the level of copper in the diets since extended meat products are not normally fortified with added trace minerals, and it was the effect of ISP substituted at different levels, on the copper naturally present in the meat, that was of primary interest.

Table 8. Levels of copper and protein in the experimental diets.

Diets	Copper		Protein (%)
	ppm	mg/g diet	
I	6.89±0.17	.00689	15.4
II	5.27±0.22	.00527	14.6
III	3.69±0.50	.00369	13.8
IV	2.09±0.12	.00209	12.6

Table 9 summarizes the data for feed consumption found in Table II of the appendix. There was a decrease in total feed consumption from a high of 3715 g for treatment I (0% ISP) to 2055.3 g for treatment III (20% ISP). This decrease in feed consumption possibly could be attributed to an increased level of fiber in the diets resulting in a feeling of satiety in the animals. Three of the chicks in treatment III died during the early stages of the feeding period. If we examine the mean feed consumption per treatment the increase seen in the total consumption is not apparent, with the decrease being continuous and significant ($p<0.05$) from treatment I to treatment IV and ranging from 265.4 g to 147.7 g.

From the copper concentration and the mean feed consumption, the amount of copper consumed per bird from each of the four treatments was calculated. There was again a decrease with an increase in ISP levels. From a high of 1.83 mg of copper consumed per bird in treatment I, there was a reduction to 0.31 mg of copper consumed per bird in the last treatment.

At one day of age, the mean value for total liver copper was 2.5±0.38 ppm. After consuming the repletion diet for two weeks, the mean copper level was reduced 24% to 1.9±0.40 ppm (Tables III and IV of appendix). This value was then taken as the basal value for determining the repletion of the liver copper from the experimental diets.

Table 10 summarizes the mean liver copper values (Table V of the appendix) before and after adjusting for basal copper value. There was a net increase in total liver copper over the basal levels for all treatments. However, there was a decrease in liver copper values from

Table 9. Total and mean feed consumption and calculated copper consumed per animal for each treatment.

Treatment	Feed Consumption		mg Cu consumed/bird
	Total (g)	Mean (g)	
I	3715.0	265.4	1.83
II	2835.3	202.5	1.08
III	2055.3	171.3	0.63
IV	2215.5	147.7	0.31

Table 10. Mean liver copper values before and after adjusting for basal copper (1.9±0.40 ppm).

Treatment	Copper (ppm)	
	Unadjusted	Adjusted
I	3.38±0.49	1.48
II	2.76±0.56	0.86
III	2.51±0.24	0.61
IV	2.93±0.92	1.03

a high of 1.48 ppm for the first treatment to 0.61 ppm for treatment III (20% ISP), followed by an increase to 1.03 ppm for treatment IV. There was a 42% reduction in total liver copper between treatments I and II and a 59% difference between treatments I and III. The difference in liver copper levels between I and IV was only 29%. The decrease between treatments II and III was of the order of 29%, while there was a net increase of 41% in the liver copper values between treatments III and IV.

Table 11 summarizes the liver weights taken from each animal (Table VI of the appendix). Using these weights, the copper per gram of liver was calculated from birds on the different treatments, as well as the availability of the copper in the four diets (Table 12). The mean concentration of liver copper was reduced in those treatments containing higher levels of ISP, ranging from 0.40 ppm copper per gram of liver for treatment I to 0.23 ppm for birds in treatment IV. In spite of the fact that the adjusted liver copper values increased from treatment III to treatment IV (Table 10), this increment was not present when the values for the amount of copper per gram of liver were examined. This was due to the larger liver weights in birds from treatment IV (Table 11).

From the calculated values per gram of liver, and the calculated copper consumed per animal, the availability was calculated (Table 12). There was an increase in the apparent availability of copper in the diets from treatments I to IV. Diet I resulted in the lowest availability even though this diet had the highest copper content the highest mean feed consumption of the four diets. The 21.86% availability is somewhat below the 30-50% range reported by various

Table 11. Summary of liver weights from the test animals.

Diets	Liver weights (g)
I	3.66±1.18
II	2.31±0.34
III	2.28±0.57
IV	4.47±0.87

Table 12. Mean copper per gram of liver and calculated availability of copper in the diets tested.

Treatment	Copper per gram liver	Availability (%)
I	0.40	21.86
II	0.37	34.25
III	0.27	42.86
IV	0.23	74.19

authors (45, 79). Treatments II and III yielded availabilities of 34.25 and 42.86%, falling within the 30-50% range. However, the apparent availability of copper in diet IV was very high (74.19%). It was of interest to note that the copper level of diet IV was below the level recommended by the NRC-NAS for chicks (4 mg/kg of feed) (61), while the levels od diets II and III were quite close to recommended levels. For this reason, animals in treatment IV were perhaps becoming deficient. Therefore, absorption of copper present in the diet would be increased by an internal mucosal mechanism in an attempt to compensate for the deficiency. This mechanism is common in living organisms and is intrinsic, in that it is triggered by deficient levels of trace minerals in the body. Johnson, et al., (45) have reported this type of response in human subjects fed copper below the recommended levels for 20 days. They reported absorptions of copper in the range of 70 to 85% in these subjects.

Apparently, the ISP in these diets had little or no negative effect on the availability of natural copper present in the diets. The observed decrease in copper levels was probably due to a dilution of the copper due to an increase in the amount of fiber and moisture in the diets. However, if the ISP had chelated copper in the meat, it is probable that availability would not have increased, as was observed.

The relationship between the levels of liver copper and the ISP levels in the diets was significant ($p<0.05$). There was a significant ($p<0.05$) combined effect of feed consumption and ISP levels. However, there was no change due only to an increase in feed consumption.

IN-VITRO STUDIES

Table 13 summarizes the values for copper from the uncooked ground beef-isolated soy protein diets obtained by the in-vitro method. Each value listed is the mean of nine observations in Table VII of the appendix. There was a decrease in the amount of copper found in the interior of the dialysis tubing from treatment I to treatment IV. This decrease might be expected since, in reality, the copper content of the raw uncooked diets decreased somewhat from treatment I to treatment IV. It should be remembered that these diets contained only meat and rehydrated ISP.

Table 13. Mean copper values obtained by the in-vitro method from uncooked diets.

Treatment	ppm	mg/g
I	0.202±0.10	0.000202
II	0.124±0.05	0.000124
III	0.096±0.05	0.000096
IV	0.091±0.04	0.000091

Table 14 contains the calculated copper content of in-vitro diets. As can be seen, there was a slight decrease in copper levels of the diets as well as a decrease in the amount of copper found in the interior of the dialysis tubing. There was a decrease in percent copper transferred, from 10.5% in the first treatment to 5.1% in the treatment with 30% ISP. Perhaps a slight binding of copper by the ISP occurred that was not apparent in the in-vivo method.

Table 14. Calculated copper content of the in-vitro diets and the percent copper passing through the semipermeable membrane.

Treatment	Copper		
	Calculated ppm	Dialyzed ppm	Transferred %
I	1.92	0.202	10.5
II	1.88	0.124	7.6
III	1.84	0.096	5.2
IV	1.80	0.091	5.1

Method of cooking the ground meat-ISP diets affected copper values obtained in the in-vitro method. the ISP-extended meat cooked by microwave gave measurable copper levels only in the unextended meat. Measurable values were not obtained from the other treatments. This could possibly be due to a loss of liquid during cooking. In spite of the fact that ISP is used in some foods for its water binding properties, there was a 28.7% cooking loss with the microwave method. This was similar to the losses of 26.4% and 29.3% as reported by Bowers, et al. (7).

The effect of pan frying on the copper values was even more dramatic. Again measurable copper values were not obtained for any of the four treatments. Likewise, it is possible that the copper contained in the meat-ISP mixture, or at least a major portion thereof, was lost in the liquid that was liberated during cooking. A cooking loss of 35.4% resulted from pan frying the meats.

ELECTROPHORESIS

In an attempt to determine the general region or site of intestinal absorption of copper and to determine the molecular weights of the proteins responsible for the binding and transport of copper released from food during the digestive process, proteins were extracted from segments of duodenum of chicks, and electrophoretic patterns determined.

Water soluble and salt soluble proteins similar photodensitometric tracings (Figure 2, 3 and 4). The molecular weights of the resulting bands ranged from 49,000 to 76,000 for the water soluble

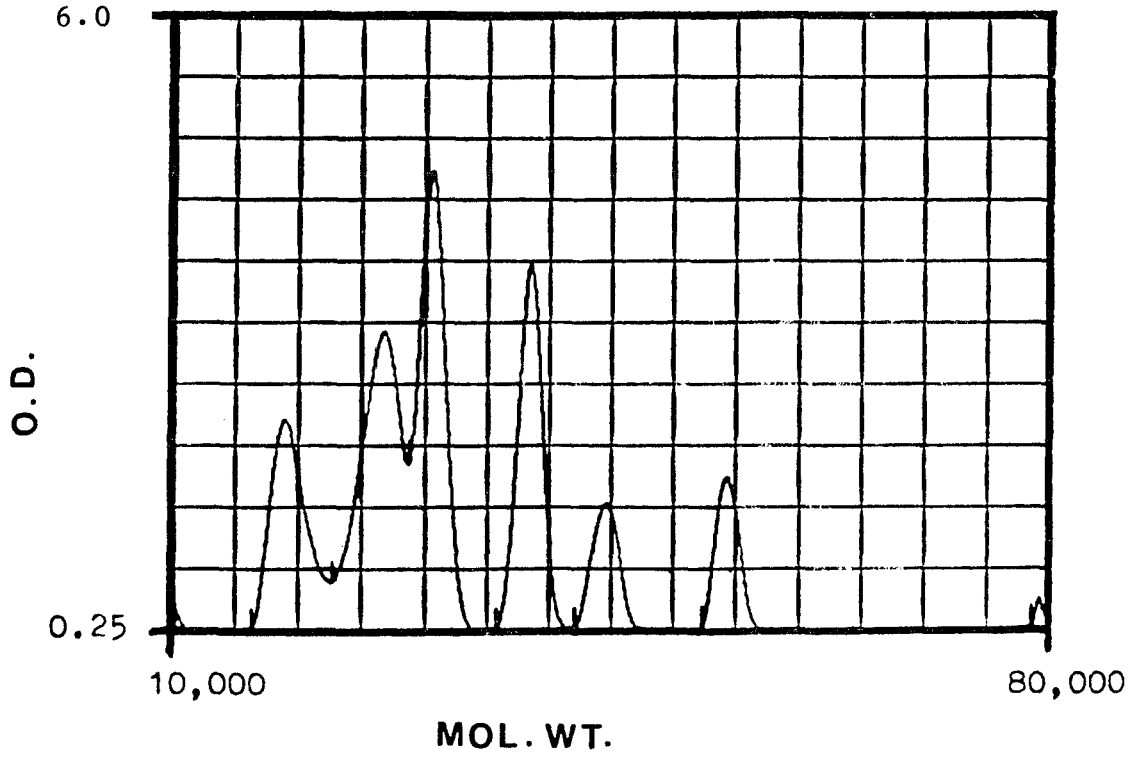

Figure 2. Photodensitometric tracing of standard protein.

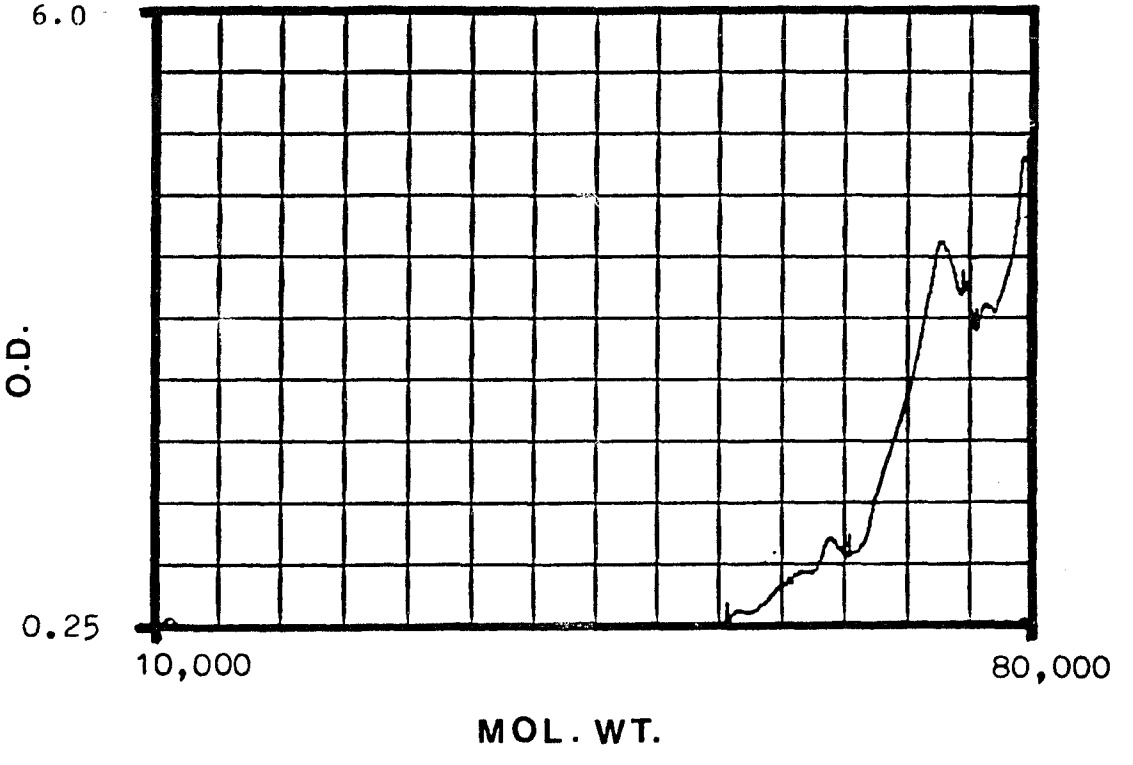

Figure 3. Photodensitometric tracing of salt soluble protein from duodenum.

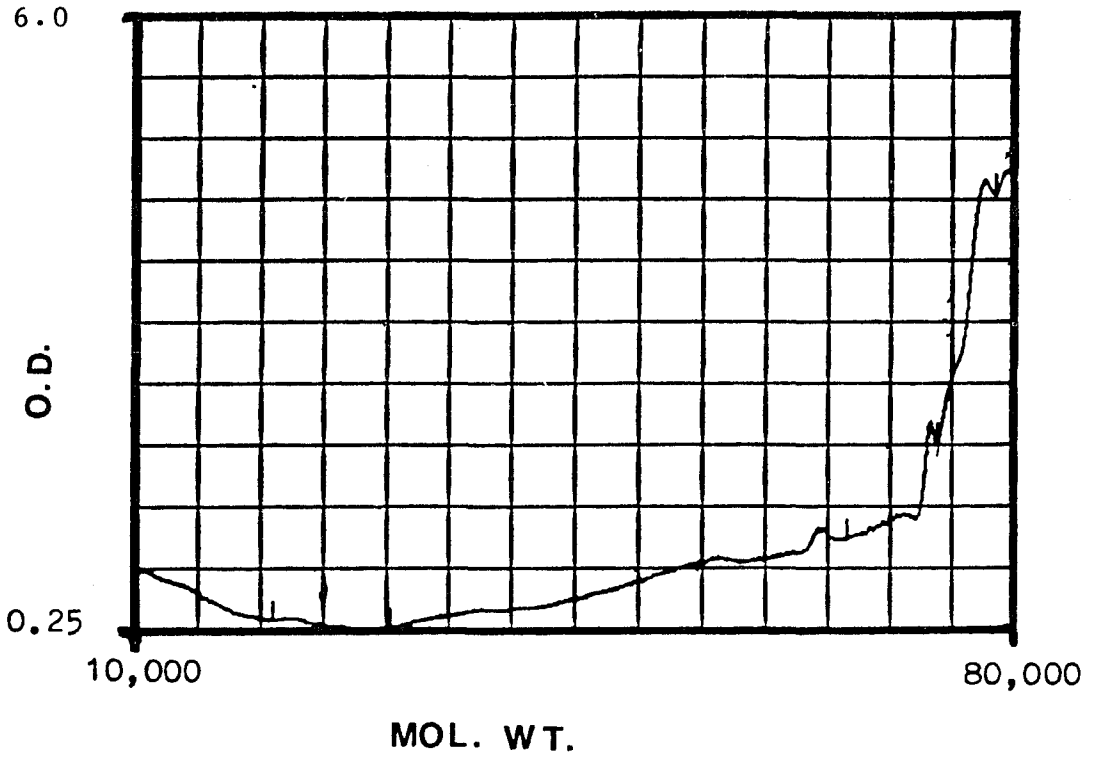

Figure 4. Photodensitometric tracing of water soluble protein from duodenum.

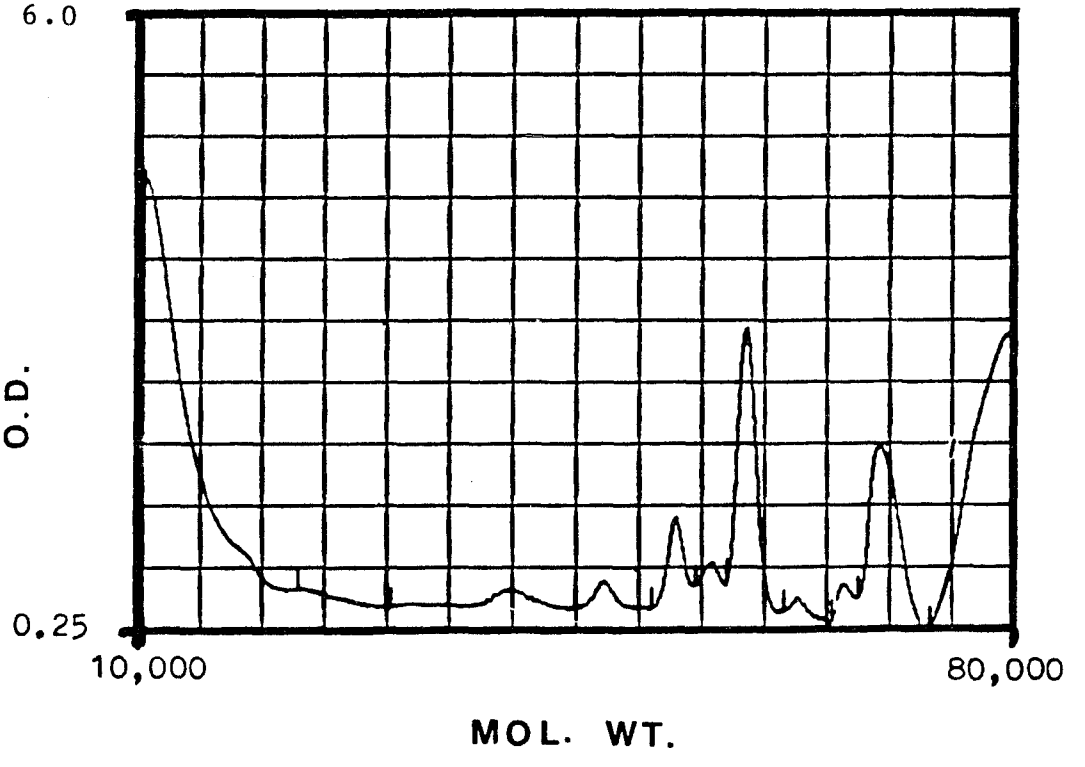

Figure 5. Photodensitometric tracing of water soluble protein from ileum.

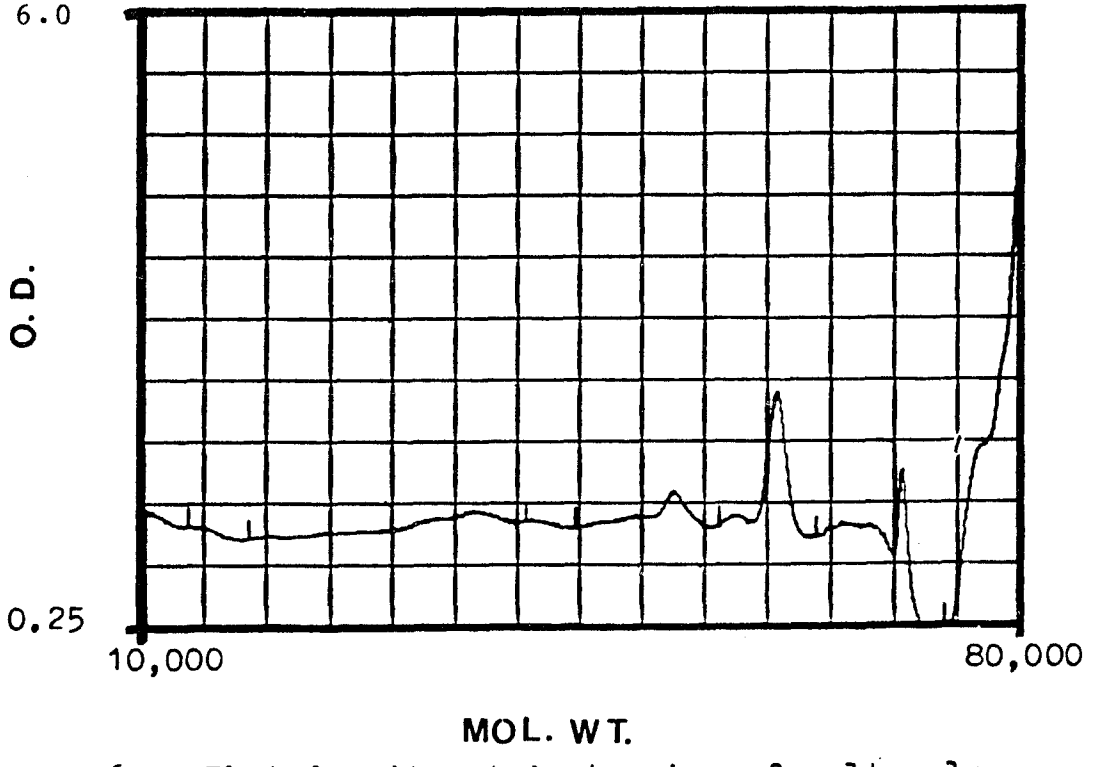

Figure 6. Photodensitometric tracing of salt soluble protein from ileum.

proteins, while the molecular weights of the salt soluble proteins ranged from 44,000 to 77,000.

Since the total copper content of the duodenum was earlier determined to be 2.861 ppm, it was thought that it would be possible to determine the protein to which the copper was bound. However, analysis of the bands did not yield measurable levels of copper. Since the extracts were not highly purified, it is possible that the copper in these bands was below detection limits of the analytical methods utilized.

Since most of the copper in foods is in the form of metallo-enzymes and copper-proteins, it probably must be liberated during digestion before being absorbed. Therefore, it might be logical to consider the possibility of absorption occurring distal to the duodenum (79). In order to investigate this possibility, both salt and water extracts from 10 cm segments of the ileum immediately following the duodenum were made and electrophoretic patterns determined.

Photodensitometric tracings of these water soluble and salt soluble proteins are shown in Figures 5 and 6. Molecular weights of the water soluble proteins ranged from 46,000 to 79,000, while the salt soluble proteins had molecular weights which ranged from 49,000 to 80,000.

Mineral analyses of the bands resulted in measurable amounts of copper associated with two of the water soluble bands and one of the salt soluble bands (Table 15). The 76,000 molecular weight water soluble protein was found to contain 0.851 ppm copper while the 70,000 molecular weight water soluble protein contained 0.451 ppm of copper.

Table 15. Molecular weights and copper content of water soluble and salt soluble proteins from the ileum.

Protein	M.W.	Copper (ppm)
Water soluble	75,660	0.851
Water soluble	69,866	0.451
Water soluble	69,23	1.100

The salt soluble protein (M.W. 69,000) seemed to have a greater affinity for copper, containing 1.100 ppm of copper.

Some authors (29) note that high levels of zinc will induce the synthesis of a high molecular weight protein which binds copper, thus making it unavailable for absorption. In the diets with the higher levels of ISP, the ratio of zinc to copper was rather high. This could possibly have created a type of artificial deficiency in the test chicks.

SUMMARY AND CONCLUSIONS

Copper depleted Single Comb White Leghorn chicks were fed test diets containing ground beef extended with 0, 10, 20 and 30% isolated soy protein for 28 days. Copper content of the test diets was less at the higher ISP content than at the 0% ISP level, and ranged from 6.89±0.17 ppm for the unextended meat (diet I) to 2.09±0.12 ppm for diet IV (30% ISP). Mean feed consumption of each diet was progressively less from a high of 265.4 g/bird with diet I to 147.7 g/bird with diet IV. Likewise, less copper was consumed per bird from diets I to IV.

Mean liver copper values showed net increases over all treatments, with a range in values from 1.48 ppm for diet I to 0.61 ppm for diet III. The liver copper values for diet IV were higher than for any of the other diets, except diet I. However, the availability of copper in each diet (mg of copper/g of liver divided by the mg of copper consumed per bird) increased continuously from diet I (21.86%) to diet IV (74.19%). The high availability calculated for treatment IV is probably due to an increased absorption of copper in the diet, even though less of the diet was consumed. Such increased absorption is common in deficient animals. Apparently, ISP had little or no negative effect upon copper in the diets, since availability increased at higher ISP levels.

Mean copper values obtained by the in-vitro method on uncooked ISP extended meats decreased from treatment I to treatment IV. Copper content of the diets decreased with an increase in ISP levels as did

the percent of copper transferred across the semipermeable membrane. It appeared that there might have been a slight binding of copper by ISP, which was not apparent in the in-vivo method.

Electrophoresis patterns obtained from water and salt extracts of chick duodenum showed high molecular weight proteins (44,000 to 77,000). However, it was not possible to obtain measurable copper in any of the bands. The water and salt soluble proteins extracted from segments of the ileum gave proteins with molecular weights ranging from 46,000 to 80,000. Two water soluble proteins (M.W. 76,000 and 70,000) had measurable levels of copper, while one salt soluble protein (M.W. 69,000) was associated with the highest levels of copper of the three proteins.

It would be interesting to continue to study intestinal proteins associated with copper absorption in order to elucidate further the mechanisms and transport of copper.

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APPENDICES

Table I. Copper content (ppm) of the test diets used in this study.

	Diets			
	I	II	III	IV
	6.792	5.019	3.120	1.981
	7.092	5.351	3.910	2.210
	6.798	5.428	4.034	2.064
$\bar{X}$	6.894	5.266	3.688	2.085
S.D.	0.1715	0.2173	0.4958	0.1158

Table II. Feed consumption of the different diets used in this study.

Treatment	Cage #	Consumption (g)
I	1	881.9
	3	1176.0
	12	1657.1
Total		3715.0
II	4	851.7
	8	1133.3
	11	850.3
Total		2835.3
III	2	721.5
	7	672.1
	9	661.7
Total		2055.3
IV	5	738.6
	6	766.8
	10	710.1
Total		2215.5

Table III. Copper content (ppm) of livers of normal one day old Single Comb White Leghorn chicks

Sample	Copper (ppm)
1	2.4
2	3.0
3	2.9
4	2.6
5	2.0
6	2.1
7	2.2
8	2.8
$\bar{X}$	2.5
S.D.	0.38

Table IV. Copper content (ppm) of livers from chicks fed a copper deficient diet for two weeks.

Sample	Copper (ppm)
1	1.751
2	1.532
3	1.879
4	1.704
5	2.960
6	2.170
7	2.162
8	2.118
9	1.872
10	1.890
11	1.507
12	1.419
13	1.737
$\bar{X}$	1.900
S.D.	0.40

Table V. Copper content (ppm) of livers taken from chicks fed the test diets during the 28 day feeding trial.

	Diets			
	I	II	III	IV
	3.14	2.74	2.61	2.37
	3.17	3.30	2.70	2.95
	2.85	2.40	2.91	3.00
	4.66	3.11	2.31	4.96
	3.73	2.00	2.54	2.24
	3.06	3.24	2.48	2.80
	3.04	1.69	2.51	1.86
	3.54	2.85	2.85	4.00
	3.06	2.78	2.20	3.56
	4.03	3.22	2.21	2.69
	3.55	3.80	2.22	1.54
	3.21	2.53	2.58	2.51
	3.34	2.40		2.54
	2.99	2.59		4.31
				2.58
$\bar{X}$	3.38	2.76	2.51	2.93
S.D.	0.49	0.56	0.24	0.92

Table VI. Weight (g) of the livers taken from chicks fed the test diets during the 28 day feeding trial.

	Diets			
	I	II	III	IV
	2.75	2.15	1.55	4.50
	3.04	1.70	1.56	3.98
	2.18	2.65	1.59	4.44
	3.91	1.89	2.28	4.80
	6.62	2.74	1.79	3.69
	3.49	2.36	2.89	3.92
	3.30	2.32	2.94	3.99
	3.45	2.76	2.25	4.91
	3.45	2.03	2.25	5.83
	2.82	2.48	2.48	5.32
	5.49	1.94	2.48	6.02
	4.21	2.38	3.26	3.82
	2.63	2.13		2.88
	3.98	2.78		5.19
				3.82
$\bar{X}$	3.66	2.31	2.28	4.47
S.D.	1.18	0.34	0.57	0.87

Table VII. Dialyzed copper (ppm) from uncooked meat-ISP diets obtained by the in-vitro method.

	Treatments			
	I	II	III	IV
	0.302	0.173	0.079	0.062
	0.320	0.086	0.091	0.169
	0.309	0.112	0.130	0.055
	0.140	0.141	0.221	0.082
	0.117	0.123	0.088	0.075
	0.112	0.074	0.000	0.160
	0.103	0.102	0.087	0.085
	0.105	0.201	0.083	0.000
	0.310	0.100	0.085	0.131
$\bar{X}$	0.202	0.124	0.096	0.091
S.D.	0.10	0.05	0.05	0.04

VITA

James L. Smith, Jr. was born in Portsmouth, Ohio on June 7, 1942. He graduated from Portsmouth East High School in 1960 and entered Ohio University where he received his B.S. degree in Zoology in 1964. He then entered the Ohio State University and received his M.S. degree in Microbiology in 1966. He served in the Peace Corps as a Volunteer in Venezuela from 1966 to 1968. After leaving the Peace Corps, he remained in Venezuela teaching Microbiology in the School of Animal Production of the Universidad de Oriente. In 1969 he married Reina Pereira and they now have six children.

In 1980 he enrolled in the graduate program in the Department of Food Science of the Louisiana State University, where he is currently a candidate for the Ph.D.

EXAMINATION AND THESIS REPORT

Candidate: James L. Smith

Major Field: Food Science

Title of Thesis: Effect of Isolated Soy Protein on the Bioavailability of Copper in Ground Beef

Approved:

Fred H Hoekstra
Major Professor and Chairman

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